Coupling of Self-activating Genes Induces Spontaneous Synchronized Oscillations in Cells

Jesus Miro-Bueno

Research Institute of the IT4Innovations Centre of Excellence, Faculty of Philosophy and Science, Silesian University in Opava, 74601 Opava, Czech Republic

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Abstract: Genetic oscillators are present in a wide range of organisms from bacteria to neurons and coordinate important biological functions. Current models of genetic oscillators are based on auto-repressed genes. In these models a gene produces a repressor protein that binds to the promoter of its own gene repressing the transcription. Different versions of these models have been studied in living organisms and for engineering synthetic clocks. Synchronization of genetic clocks based on this model has also been studied. However, genes with positive feedbacks are also present in natural and synthetic genetic clocks. These self-activating genes provide robustness and frequency tuning to genetic clocks. In this paper we show a novel role of self-activating genes. We demonstrate that the coupling of self-activating genes by small molecules in a cell population produces synchronized oscillations. Our model could be useful for engineering new robust multicellular clocks and better understanding of natural genetic oscillators.

1 INTRODUCTION

Synchronization is an essential process for the proper functioning of living organisms from bacteria to mammals. Synchronization is important to coordinate the gene expression of bacteria populations in order to act in unison (Waters and Bassler, 2005; Ng and Bassler, 2009). In mammals, cells produce reliable and synchronized oscillations in the gene expression which control important functions such as metabolism, signalling and cell cycle (Mohawk et al., 2012; Welsh et al., 2010). Moreover, cell populations that oscillate synchronously have been implemented in the laboratory in the last few years (Danino et al., 2010; Prindle et al., 2014). In the above cases, the presence of feedback loops is ubiquitous in genetic networks. Feedback loops have been important in the design and implementation of initial synthetic genetic devices. For example, the genetic toggle switch is a synthetic device constructed in *Escherichia coli* bacteria (Gardner et al., 2000). This genetic network has two stable states, and it is possible to flip from one to the other induced by external signals. The toggle switch consists basically of two genes with two repressible promoters. Each gene encodes a different repressor protein, which is able to bind to the promoter of the other gene and inhibits its expression. Other example is the so-called repressilator, that is the first synthetic genetic oscillator constructed. The repressilator is also a synthetic device implemented in *Escherichia coli* (Elowitz and Leibler, 2000). It is a genetic network that can produce an oscillatory behaviour. It consists of three genes with three repressible promoters. The genes are connected to each other forming a ring. As in the toggle switch, each gene encodes a repressor protein that is able to bind to the promoter of the next gene and inhibits its expression. The result of these three repressive interactions is the creation of a negative feedback loop with delay, which is known to produce sustained oscillations (Novák and Tyson, 2008). Several synthetic genetic oscillators, based on different designs, have also been implemented after the repressilator (Atkinson et al., 2003; Fung et al., 2005; Stricker et al., 2008; Balagaddé et al., 2008; Tigges et al., 2009; Toettcher et al., 2010; Kim and Winfree, 2011; Montagne et al., 2011; Weitz et al., 2014).

Genes often express transcription factors that regulate their own transcription rates in genetic networks. This behaviour of the genes produces feedback loops. If a gene expresses a transcription factor that increases its own transcription rate, a positive feedback loop is created. In contrast, when a gene expresses a transcription factor that decreases its own transcription

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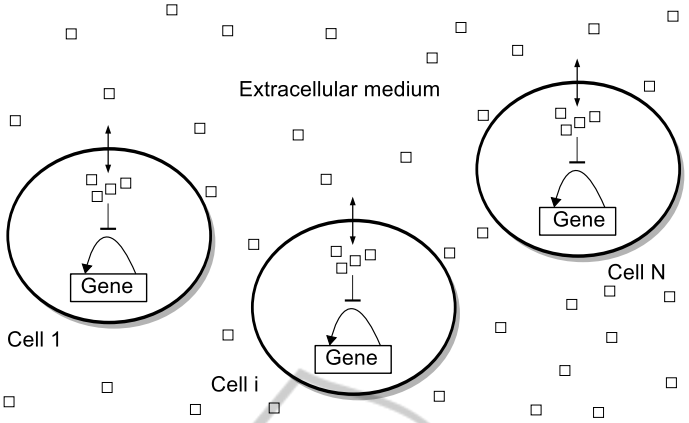

Figure 1: The model. Population of cells in which each cell contains a self-activating gene. External small molecules (squares) can diffuse across the membrane and couples the self-activating genes. The small molecules inhibit the proteins in the positive feedback loop.

rate, a negative feedback loop is created. A gene with positive feedback loop can produce bistability (Keller, 1995; Smolen et al., 2000; Becskei et al., 2001; Ferrell Jr., 2002; Mitrophanov and Groisman, 2008). This means that two steady states are possible in the system. In one state there is a high number of molecules, for example proteins, and in other state there is a low number of molecules. Positive feedbacks are also present in many natural genetic clocks (Reppert and Weaver, 2002; Gallego and Virshup, 2007; Purcell et al., 2010; Lenz and Søgaard-Andersen, 2011; O'Brien et al., 2012). In genetic oscillators, it seems that the negative feedback is mainly involved in generating oscillations, whereas the positive feedback allows tuning the oscillations without changing the amplitudes (Tsai et al., 2008), contributes to increase the robustness of these oscillations (Vilar et al., 2002; Tsai et al., 2008) and could provide robust adaptation to environmental cycles (Rand et al., 2004; Mondragón-Palomino et al., 2011). Models of genetic clocks involving negative and positive auto-regulated genes have been studied in the last few years (Barkai and Leibler, 2000; Smolen et al., 2001; Hasty et al., 2001; François, 2005; Guantes and Poyatos, 2006; Rodrigo et al., 2007; Hong et al., 2008; Conrad et al., 2008; Krishna et al., 2009; Nandi et al., 2009; Munteanu et al., 2010). In these models the positive feedback loops increase the expression of repressor genes. Moreover, positive auto-regulated genes with simple negative interactions can produce reliable oscillations (Miró-Bueno and Rodríguez-Patón, 2011). Beyond increasing robustness, studies about the role of self-activating genes in the direct production of oscillations are needed to understand better cellular clocks. Coupling of negative auto-regulated genes is the usual way for producing synchronized oscillations in the study and modelling of synthetic gene clocks (McMillen et al., 2002; Garcia-Ojalvo et al., 2004). Here, we present and study a new model to elucidate the role of self-activating genes in the production of rhythms in cell populations. We show that the coupling of self-activating genes by transmembrane movement of small molecules, such as metabolites, produces synchronized oscillations in a cell population.

2 MODEL

The model is a population of cells in which each cell contains a self-activating gene (Fig. 1). We consider the simplest form of a self-activating gene without protein cooperation or multimers (Vilar et al., 2002; Miró-Bueno and Rodríguez-Patón, 2011). This gene expresses a protein that produces a positive feedback loop. The protein binds to the promoter of its own gene and increases the transcription rate. An external small molecule is in charge of coupling the self-activating genes. This small molecule enters into the system at constant rate. This molecule can pass through the membrane. We do not assume an specific way, the only condition is that the molecule can pass in both directions, from outside to inside of cells and vice versa. Inside cells, the small molecule can inhibit the protein that produces the positive feedback. Then, the protein can not bind to its promoter when attached to the small molecule. At the same time, the small molecule can not go outside the cell because it is attached to the protein. When the protein is degraded the small molecule is released and can bind to other protein or leave the cell crossing the membrane. The model is described by $12N+2$ biochemical reactions, where N is the number of cells. The chemical reac-

tions that describe the gene with positive feedback in each cell i are as follows:

$$\begin{aligned}
&\text{Activation:} && G_i + Ai \xrightarrow{k_1} G_i^a \\
&\text{Deactivation:} && G_i^a \xrightarrow{k_{-1}} G_i + A_i \\
&\text{Slow transcription:} && G_i \xrightarrow{k_2} G_i + M_i \\
&\text{Fast transcription:} && G_i^a \xrightarrow{k_3} G_i^a + M_i \\
&\text{mRNA degradation:} && M_i \xrightarrow{k_4} \phi \\
&\text{Translation:} && M_i \xrightarrow{k_5} M_i + A_i \\
&A \text{ degradation:} && A_i \xrightarrow{k_6} \phi
\end{aligned} \tag{1}$$

where G_i denotes the gene without A_i bound to its promoter, M_i denotes mRNA transcribed from G_i, A_i denotes the activator protein translated from M_i, G_i^a denotes the gene with A_i bound to its promoter. The description of the rates is as follows: k_1 is the binding rate of A_i to the promoter of G_i, k_{-1} is the unbinding rate of A_i from the promoter of G_i, k_2 is the basal transcription rate, k_3 is the activated transcription rate, k_4 is the degradation rate of M_i, k_5 is the translation rate and k_6 is the degradation rate of A_i.

On the other hand, the chemical reactions that describe the dynamics of the small molecule are as follows:

$$\begin{aligned}
&\text{Complex creation:} && S_i + A_i \xrightarrow{k_7} C_i \\
&\text{Complex decay:} && C_i \xrightarrow{k_8} S_i \\
&S \text{ entry:} && S \xrightarrow{k_9} S_i \\
&S_i \text{ exit:} && S_i \xrightarrow{k_{10}} S \\
&S_i \text{ degradation:} && S_i \xrightarrow{k_{11}} \phi \\
&S \text{ creation:} && \phi \xrightarrow{k_{12}} S \\
&S \text{ degradation:} && S \xrightarrow{k_{13}} \phi
\end{aligned} \tag{2}$$

where S_i denotes the small molecules inside cell i, C_i denotes S_i bound to protein A_i and S denotes the small molecules in the extracellular medium. The description of the rates is as follows: k_7 is the binding rate of S_i to A_i, k_8 is the decay rate of C_i into S_i, k_9 is the entry rate of S into the cell, k_{10} is the exit rate of S_i from the cell i, k_{11} is the degradation rate of S_i, k_{12} is the synthesis rate of S and and k_{13} is the degradation rate of S. We perform stochastic simulations of the model since it is more realistic than deterministic simulations and takes into account the random nature of chemical reactions (Gillespie, 1977). We have chosen typical parameter values to produces circadian rhythms (24-hour period) due to its relevance in biological systems (Vilar et al., 2002; Miró-Bueno and

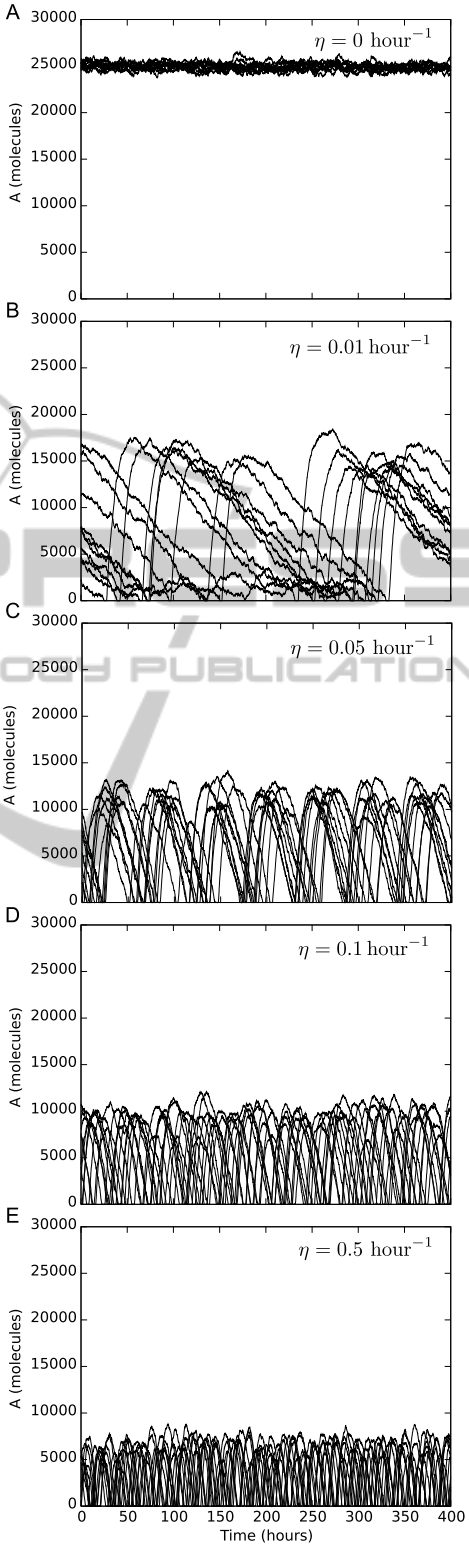

Figure 2: Production of oscillations. The number of proteins start oscillating in cells when the small molecule can diffuse across the membrane. These oscillations are unsynchronized.

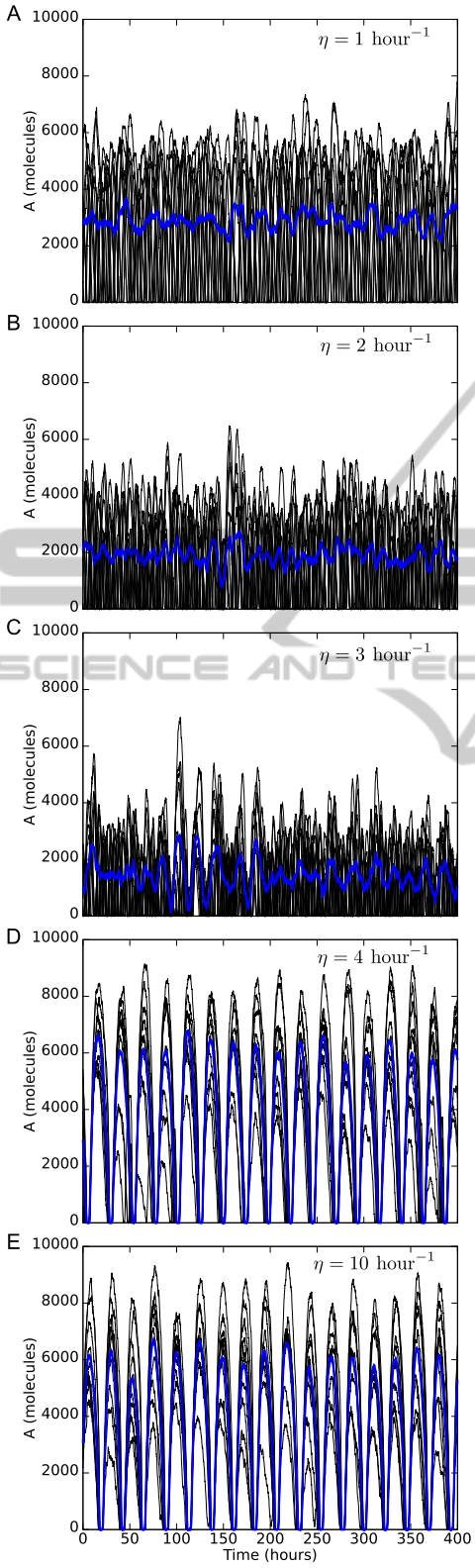

Figure 3: Synchronization of oscillations. Increasing the diffusion rate across the membrane produces synchronized oscillations. Blue lines represent the average.

Rodríguez-Patón, 2011). The values of the rates are: $k_1 = 1$ molecule^{-1} hour^{-1}, $k_{-1} = 50$ hour^{-1}, $k_2 = 50$ hour^{-1}, $k_3 = 500$ hour^{-1}, $k_4 = 10$ hour^{-1}, $k_5 = 50$ hour^{-1}, $k_6 = 0.1$ hour^{-1}, $k_7 = 0.5$ molecule^{-1} hour^{-1}, $k_8 = 2.6$ hour^{-1}, $k_9 = \eta$, $k_{10} = \eta$, $k_{11} = 1$ hour^{-1}, $k_{12} = 50$ molecule hour^{-1} and $k_{13} = 1$ hour^{-1}. We have chosen random initial conditions for each simulation. Each type of molecule was randomly set to be between 0 and 1,000 molecules. For the number of genes we choose $G_i = 1$ molecule. The first 100 hours of transient behaviour were discarded. In this model, the synchronization of the cell population depends on the diffusion rate across the membrane (η). The small molecules can pass through the cell membrane from extracellular to intracellular space and vice versa. In our model, this means that the rates k_9 and k_{10} are the same, i.e., $\eta = k_9 = k_{10}$. We assume that diffusion of the small molecule in the extracellular medium reaches the equilibrium fast in comparison with typical biochemical reactions such as transcription, translation or degradation.

3 RESULTS AND DISCUSSION

The dynamics for ten coupled cells are shown in Figs. 2 and 3. In Fig. 2 we show that the number of proteins start oscillating in cells when the small molecule can diffuse across the membrane. When η is 0 there is not coupling, and each cell in the population expresses the protein until its maximum value 25,000 molecules, i.e., a fixed point is reached (Fig. 2A). When η increases from 0 to 0.01 hour^{-1} the number of proteins oscillates in each cell (Fig. 2B). This oscillatory behaviour appears in each cell due to the negative interaction between the small molecule and the positive feedback loop (Miró-Bueno and Rodríguez-Patón, 2011). The negative interaction is the inhibition of proteins by small molecules. The oscillations involved two stages. In the first one, the small molecules are accumulated in the cells, due to the positive feedback is at its maximum strength. In the second stage the small molecules are released or degraded in cells, due to the positive feedback is in its minimum strength. The amplitude is about 15,000 molecules and the period is about 150-200 hours. Each cell in the population produces oscillations but these oscillations are not synchronized. If the rate η is increased the amplitude and period of the oscillations are decreased (Figs. 2C,D,E).

In Fig. 3 we show that increasing the diffusion rate η produces synchronized oscillations. When $\eta = 1$ hour^{-1} the oscillations are not synchronized (Fig. 3A). In this case, the diffusion rate across the

membrane is not fast enough to induce synchronized oscillations in the cell population. When η is increased the oscillations are still not synchronized and the amplitude is decreased (Figs. 3B,C). This reduction in the amplitude of the oscillations is because proteins are inhibited by small molecules. When η = 4 hour^{-1} the oscillations are synchronized with a period of about 24 hours due to small molecules can diffuse fast enough between the cells (Fig. 3D). The amplitude in the synchronized oscillations is higher than the oscillations showed in Fig. 3A. If the vale of η is increased to 10 hour^{-1} (Fig. 3E), the dynamics of the oscillations is the same as in Fig. 3D.

In Fig. 4 we show that the synchronization of the oscillations in the cell population increases when the value of η is raised. Specifically, we show that there is a transition from unsynchronized to synchronized oscillations as a function of the diffusion rate of the small molecule. We use the so-called order parameter (r_{sync}) to measure the degree of synchrony over a time interval (Garcia-Ojalvo et al., 2004). The r_{sync} for the proteins A is the ratio of the variance of the mean number of A in all the cells to the mean variance of each cell in a time interval:

$$r_{sync} = \frac{\langle \overline{A}^2 \rangle - \langle \overline{A} \rangle^2}{\frac{1}{N}\sum_{i=1}^{N}(\langle A_i^2 \rangle - \langle A_i \rangle^2)}, \qquad \overline{A} = \frac{1}{N}\sum_{i=1}^{N} A_i, \tag{3}$$

where brackets denote time average. The value of r_{sync} is between 0 and 1, where 0 corresponds to unsynchronized oscillations and 1 corresponds to perfect synchronized oscillations. The oscillations are unsynchronized when η is lower than about 3 hour^{-1}. In this case, the value of r_{sync} is lower than 0.1. However, when η is increased until about 3.5 hour^{-1} the oscillations spontaneously synchronized. In this case, the value of r_{sync} is about 0.7. And if the value of η is higher than 3.5 hour^{-1} the value of the order parameter r_{sync} approximates to 1. The coupling of the self-activating genes by a small molecule produces the oscillations first and then the transition from unsynchronized to synchronized oscillations. This type of transition in a coupled population of oscillators was predicted by Winfree (Winfree, 2002). For example, this transition has been found in a cell population with coupled genetic clocks (Garcia-Ojalvo et al., 2004). The difference is that we do not have genetic clocks in our model if the coupling agent is not present. In our model the small molecules are the coupling agents and also are directly involved in the production of oscillations. Another difference is that our model does not involve genes with negative feedback loops. Our

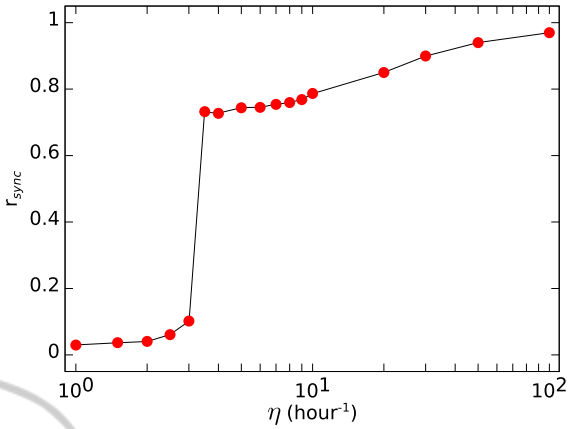

Figure 4: Order parameter as a function of the diffusion rate of the small molecule across the membrane. Computed time for each point: 2,500 hours (1,000 hours for η= 20, 30, 50 and 100 hour^{-1}).

study shows that there is an alternative possibility for a cell population to produces synchronized oscillations. We speculate that both auto-repressed and self-activating genes can participate in the production of synchronized oscillations together or separately. It is known that a positive feedback loop can provide robustness to molecular noise in a genetic clock driven by a gene with negative feedback. We hypothesize that the presence of self-activating genes could also increase the robustness to failures in a cell population that produces synchronized rhythms. A future work is study how self-activating genes can produce synchronized oscillations with simple conditions if the genes with negative feedback failed. Another future work is to study the behaviour of the cell population when the synthesis rate of the small molecules is not a constant value.

4 CONCLUSIONS

We have found that the coupling of self-activating genes by small molecules in a cell population can produce synchronized oscillations. This finding is a new role of self-activating genes. In our model, the small molecules are the coupling agents and are also directly involved in the production of oscillations. We have found a transition from unsynchronized to synchronized oscillations as a function of the diffusion rate of the small molecule. This behaviour could be interesting for engineering new synthetic multicellular clocks and for better understanding the role of self-activating genes in genetic clocks.

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